nuclear pools of FH, whose tumor suppressor functions rely on DNA damage repair and stabilization of HIF-1α-signaling that induces pseudohypoxia.

References

[1] A. Klaus, C. Polge, S. Zorman, Y. Auchli, R. Brunisholz, U. Schlattner, J. Proteomics 75 (2012) 3304–3313.
[2] A. Brückner, C. Polge, N. Lentze, D. Auerbach, U. Schlattner, Int. J. Mol. Sci. 10 (2009) 2763–2788.

doi:10.1016/j.bbabio.2012.06.233

11P8

Mitochondrial potassium channels in *Dictyostelium discoideum*

M. Laskowski[1], A. Kicinska[2], W. Jarmuszkiewicz[2], A. Szewczyk[1]
[1]*Nencki Institute of Experimental Biology, Laboratory of Intracellular Ion Channels, Warsaw, Poland*
[2]*Laboratory of Bioenergetics, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland*
E-mail: m.laskowski@nencki.gov.pl

Mitochondria are crucial not only in energy metabolism but also in regulation of cell senescence and apoptosis. The strict control of inner mitochondrial membrane permeability and selective ion transport is essential for mitochondria functioning. Potassium ion homeostasis is an important process for mitochondrial optimal functioning. Potassium channels such as ATP-regulated, large conductance calcium activated and voltage dependent channels were observed in inner mitochondrial membrane in various mammalian tissues. Recently, we have identified potassium channels in inner mitochondrial membrane of potato *Solanum tuberosum* and *Acanthamoeba castellanii*. Currently we characterize mitochondrial potassium channels from one of Dictyostelium species. It is commonly used as a model organism to study cell differentiation, metabolism and programmed cell death. Preliminary experiments are focused on biophysical and pharmacological characterization of mitochondrial ion channels. Purified inner mitochondrial membranes (submitochondrial particles) were reconstituted into planar lipid bilayer. To form model membranes asolectin from soybean mixture of phospholipids was used. We observed two types of potassium selective ion channels in submitochondrial particle samples: a large- and small-conductance channels. Experiments were performed both in gradient solution 50/150 mM KCl (cis-trans) and in symmetrical solution 150/150 mM KCl at voltages from −50 to 50 mV. Regulation of the channel activity by divalent cations such as Ca^{2+} and Mg^{2+} was explored. Additionally, interaction of the ATP with mitochondrial potassium channels was characterized. The knowledge on mitochondrial ion channels may contribute to understanding molecular mechanism of *Dictyostelium discoideum* functioning.

This work was supported by Polish Mitochondrial Network MitoNet.pl.

doi:10.1016/j.bbabio.2012.06.234

11P9

Reactive oxygen species in proinflammatory response of endothelial cells

A. Lukasiak[1], A. Zgrzywa[2], A. Wrzosek[2], A. Szewczyk[2]
[1]*Department of Biophysics, Warsaw University of Life Sciences SGGW, 159 Nowoursynowka St., 02-776 Warsaw, Poland*
[2]*Laboratory of Intracellular Ion Channels, Nencki Institute of Experimental Biology, 3 Pasteura St., 02-093 Warsaw, Poland*
E-mail: agnieszka_lojek@sggw.pl

Endothelium is a thin layer of cells lining all cardiovascular system. It plays crucial role in such diseases as atherosclerosis, hypertension and diabetes. It is well documented that the inflammation is responsible for these diseases, however very little is known about the role of mitochondria in development of proinflammatory state. It is known that reactive oxygen species (ROS) produced by mitochondria can be involved in the proinflammatory process.

As a model of our study we used endothelial immortalized cell line EA.hy 926 and as a marker molecule the ICAM-1 (intracellular adhesion molecule 1) expression was measured using flow cytometry method. The inflammation was induced by cytokine TNF-α (tumor necrosis factor α). We examined the role of reactive oxygen species (ROS) in the proinflammatory process using fluorescent probe DCF-DA. The main source of ROS production in cells is mitochondria, therefore we checked the effect of rotenone, the complex I of respiratory chain inhibitor, on ROS level in EA.hy 926 cells.

In our study TNF-α caused time and dose dependent increase of ICAM-1 from hardly detected residual level. Additionally our results show that TNF-α increases ROS production in EA.hy 926 cells in dose dependent manner. Rotenone was ineffective in changing the ROS production level in EA,hy 926 cells. Our results related to rotenone are slightly different to literature data that suggests that different cellular models can response to rotenone in different ways.

This work was supported by the European Union from the resources of the European Regional Development Fund under the Innovative Economy Programme (POIG.01.01.02-00-069/09).

doi:10.1016/j.bbabio.2012.06.235

11P10

NCLX, but not Letm1, mediates matrix Ca^{2+} extrusion and modulates the mitochondrial redox state during HeLa cell stimulation

Umberto De Marchi[1,2], Jaime Santo-Domingo[2], Israel Sekler[3], Andreas Wiederkehr[1], Nicolas Demaurex[2]
[1]*Nestlé Institute of Health Sciences, EPFL Campus, Bâtiment G, Quartier de l'Innovation, CH-1015 Lausanne, Switzerland*
[2]*Department of Cell Physiology and Metabolism, University of Geneva, rue Michel-Servet, 1, CH-1211 Genève, Switzerland*
[3]*Department of Physiology, Ben-Gurion University of Negev, Beer-Sheva, 84105, Israel*
E-mail: umberto.demarchi@rd.nestle.com

Mitochondria sense and shape intracellular Ca^{2+} signals, acting as a cell signaling hub. The uptake of Ca^{2+} into the mitochondrial matrix activates intermediary and energy metabolism, and Ca^{2+} extrusion mechanisms ensure that this Ca^{2+} signal is transient. After a long quest, the proteins promoting Ca^{2+} uptake and release have been discovered: a mitochondrial Ca^{2+} uniporter was shown to mediate Ca^{2+} uptake, and two ion exchangers, NCLX and Letm1, were proposed to exchange Ca^{2+} against Na^{+} or H^{+} respectively.

To relate mitochondrial Ca^{2+} extrusion to mitochondrial function, we have manipulated the expression levels of NCLX and Letm1 and measured by single cell imaging their impact on matrix Ca^{2+}, matrix redox state, and NAD(P)H concentration evoked by Ca^{2+} mobilizing agonists.

We find that the histamine stimulated mitochondrial Ca^{2+} rise is highly variable in individual HeLa cells. The rate of Ca^{2+} extrusion is a function of this amplitude, being highest for large matrix Ca^{2+}